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Abstract

Purpose of review

Inflammatory bowel diseases (IBDs) develop as a result of a combination of genetic predisposition, dysbiosis of the gut microbiota, and environmental influences. Here, we describe an example of how Caspase recruitment domain 9 (CARD9), one of the numerous IBD susceptibility genes, participate to colitis susceptibility by shaping gut microbiota to produce tryptophan metabolites.

Recent findings

Recent study showed that *Card9*$^{-/-}$ mice are more susceptible to colitis as a result of impaired interleukin 22 (IL-22) signaling pathway. Furthermore, aryl hydrocarbon receptor (AhR) ligands from tryptophan metabolism by the gut microbiota participate to intestinal homeostasis by inducing production of IL-22 by intestinal immune cells. These data suggest an interaction between CARD9 and the ability of gut microbiota to produce AhR ligands.

Summary

The microbiota from *Card9*$^{-/-}$ mice fails to metabolize tryptophan leading to defective AhR activation which contributes to the susceptibility of mice to colitis by decreased IL-22 production. These effects were abrogated in the presence of AhR agonist. Reduced production of AhR ligands is also observed in the microbiota from individuals with IBD, particularly in those with *CARD9* risk alleles associated with IBD. Correcting impaired microbiota functions, such as ability to produce AhR ligands, is an attractive strategy in IBD.

Keywords: CARD9, IBD, microbiota, tryptophan.

INTRODUCTION

The gastro-intestinal tract is a complex milieu in which coexist host epithelial and immune cells, a wide array of microorganisms and molecules from food or microbiota metabolism. A functional intestinal barrier is essential to maintain the balance between health and disease. This is accomplished by a tightly regulated process involving multiple cell types. The single layer of epithelial cells forms a physical barrier between the intestinal lumen and the host's lamina propria (LP). Specialized intraepithelial lymphocytes (IELs), such as TCR γδ cells, are located between epithelial cells and represent a critical first line of defense. In the LP, a variety of immune cells are present (B cells, T cells, macrophages, dendritic cells, and innate lymphoid cells (ILCs)) that participate in the maintenance of intestinal homeostasis. The intestinal immune system plays a crucial role in shaping the microbiota so it can be tolerated by the host.

The human intestinal microbiota is a complex ecosystem composed of more than 10^{11} microorganisms per gram of feces. It plays a crucial role in wide variety of vital functions such as digestion, immune system development, resistance against intestinal pathogens, etc. Indeed, germ-free (GF) mice have defects in intestinal immune system development and function, including in development of gut-associated lymphoid tissue and production of Immunoglobulin A [1*]. Moreover, metabolites derived from commensal bacteria, as the short-chain fatty acids (SCFA), the polyamines and the aryl hydrocarbon receptor (AhR) ligands may regulate immune cells functions via indirect and direct mechanisms [1*]. *Lactobacilli* spp. can metabolize dietary tryptophan in AhR ligands that induce IL-22 production by ILC leading to production of antimicrobial peptides by intestinal epithelial cells which provide resistance for opportunistic pathogens such as *Candida albicans* [2].

In correlation, tryptophan-free diet in mice decreases the production of intestinal antimicrobial peptides that affects the gut microbiota composition and diversity, suggesting that tryptophan metabolism by the gut microbiota play an important role in mucosal homeostasis [3]. Therefore, the complex bilateral interaction between the host and its microbiota has a crucial role in human health. In some circumstances, the tolerance toward the intestinal microbiota is broken, leading to inappropriate immune response and intestinal or extra-intestinal inflammation. This is the case in inflammatory bowel diseases (IBD).

Inflammatory bowel disease (IBD)

IBD include Crohn's disease (CD) and ulcerative colitis (UC) and are characterized by pathological inflammation of the digestive tract. In CD, inflammation affects the entire digestive tract while only the rectum and colon are affected in UC. These diseases have periods of active illness followed by periods of remission whose duration and frequency are patient dependant. IBDs appear most often between the age of 20 and 30 years old. These pathologies modify for the long-term the health-related quality of life of patients and currently these chronic diseases do not have curative treatment. The highest occurrence of IBD is found in the developed countries of North America and Europe but IBD has also emerged in newly industrialized countries as well as Asia and South and Central America. The pathogenesis of IBD is unknown but involves an activation of the gastro-intestinal immune system toward the gut microbiota in genetically susceptible hosts and under the influence of environment. As pointed out above, the composition of the intestinal microbiota affects the host as a whole. In recent studies, anti- or pro-inflammatory microorganisms have been identified: in the mice, segmented filamentous bacteria induce

T helper 17 (Th17) cells in the small intestine which have pro-inflammatory effects [4*]. Other bacteria like *Bacteroides fragilis* [5] and *Feacalibacterium prausnitzii* [6,7*] have anti-inflammatory effects mediated by metabolites production and/or regulatory T cells recruitment. Thus, loss of the fragile equilibrium within this complex ecosystem termed dysbiosis often characterized by a decreased biodiversity, overgrowth of potentially harmful microorganisms and disappearance of protective ones can eventually trigger numerous pathologies, including IBD [8*,9*]. However, it is still unclear whether dysbiosis is a cause or a consequence of these diseases. In addition of the dysbiosis, genetic factors are also suspected in the IBD pathogenesis. More than 160 IBD susceptibility loci have been identified and among them several in the gene CARD9 [10*].

Caspase Recruitment Domain 9 (CARD9)

CARD9 is signaling protein that is highly expressed in myeloid cells and particularly in macrophages and dendritic cells. It is an adapter protein playing a central role for the integration of signals downstream of Pattern Recognition Receptors (PRRs). CARD9 play a major role in the sensing of mycobacteria, bacteria, fungi and virus via different receptors (Mincle, TLR, NOD2 and Dectin respectively). Downstream of CARD9, several pathways are involved including NF-kB and p38/JNK leading to cytokines production and participating to the elimination of the detected microorganisms. Card9 is thus a key adapter protein for innate immunity toward a wide range of microorganisms including many intestinal commensals and pathogens [11,12,13*,14]. We showed in a previous study that Card9 knockout mice (*Card9*^{-/-}) are more susceptible to dextran sulfate sodium (DSS) – induced colitis which is used to mimic IBD in mice [12]. *Card9*^{-/-} mice exhibited an

impaired immune response with defective expression of IL-6, IL-17, IFN-γ and IL-22 in the colon [12]. IL-22 is involved in mucosal wound healing and it mediates innate antimicrobial resistance in mice by inducing production of antimicrobial peptides, such as Reg3γ and Reg3β, by intestinal epithelial cells [15*,16]. Moreover, we have recently showed that intestinal epithelial cell proliferation is reduced and apoptosis is increased after DSS-induced colitis in $Card9^{-/-}$ mice [17*]. In these mice, we also observed a decreased colonic expression of Reg3γ and Reg3β and an alteration of the bacterial and fungal microbiota [17*]. The major role of IL-22 and some of its target genes Reg3γ and Reg3β in the response to bacterial infections suggested that Card9$^{-/-}$ gut microbiota might be deeply impacted.

Impact of the *Card9$^{-/-}$* microbiota in the susceptibility to colitis

Using the linear discriminant analysis effect size (LEfSe) pipeline, we observed several differences in the composition of the fecal bacterial microbiota at baseline in WT and $Card9^{-/-}$ mice, including decreases in *Adlercreutzia* (genus), *Allobaculum* (genus), and *Lactobacillus reuteri* in $Card9^{-/-}$ mice [17*]. These data confirmed that CARD9 plays an important role in shaping the microbiota but raises questions about the specific role of the microbiota in the hyper-susceptibility to colitis that is observed in $Card9^{-/-}$ mice. To address this question, we colonized GF WT mice with the microbiota of WT (WT→GF) or $Card9^{-/-}$ ($Card9^{-/-}$→GF) mice and exposed them to DSS. The transfer of $Card9^{-/-}$ microbiota in GF WT mice was sufficient to recapitulate the phenotype observed in $Card9^{-/-}$ mice. Indeed, we observed an increased susceptibility to colitis with decreased proliferation and increased apoptosis in intestinal epithelial cell of $Card9^{-/-}$→GF mice. Transcriptomic

analysis in the colon showed also a down-expression of IL-22, Reg3γ and Reg3β in *Card9^{-/-}*→GF mice. In these animals, decreased production of IL-22 in the colon and in the mesenteric lymph nodes was also observed. No differences in IL-22 amounts were observed in splenocytes, suggesting a gut-limited defect in *Card9^{-/-}*→GF mice [17*]. Several sources of IL-22 have been identified in the gut, including ILCs, natural killer cells, T helper 17 (Th17) and Th22 cells, TCR γδ cells, and lymphoid tissue inducer (LTi) cells [15*,18]. Flow cytometry analysis of these different cells demonstrated that the microbiota of *Card9^{-/-}* mice is defective in inducing IL-22 production by Th22, NKp46^{+} ILCs, LTi cells, and CD3^{−}CD4^{−}NKp46^{−} cells in the colon, leading to increased susceptibility to colitis [17*].

Tryptophan metabolism is impaired in *Card9^{-/-}*→GF mice

Recent data have suggested that tryptophan catabolites generated by the microbiota metabolism have a role in mucosal immune responses via the aryl hydrocarbon receptor (AhR) by modulating production of IL-22 [2,19]. AhR is a ligand-activated nuclear transcription factor widely expressed in the body and evolutionarily conserved from invertebrates onwards [20]. In the absence of a ligand, AhR is retained in an inactive complex in the cytoplasm but after ligand binding it translocates into the nucleus where it binds genomic regions containing the dioxin response element (DRE) that regulates expression of target genes. For many years AhR was almost exclusively studied for its role in mediating the toxicity of xenobiotics, and notably of a side product in industrial organic synthesis of herbicides, 2,3,7,8-tetrachlorodibenzo-p-dioxin. Recently, AhR signaling has been shown to be a key player of the immune response, particularly at barrier sites such as

the skin and the intestinal mucosa [20,21**]. Tryptophan can be metabolized either by the gut bacteria into indole derivatives, such as indole-3-acetic acid (IAA) or by host cells into kynurenine (Kyn) via indoleamine 2,3-dioxygenase 1 (IDO1) [2,21**]. Indole derivatives are AhR ligands known to promote local IL-22 production by immune cells [1*,21**]. To determine whether the modulation of AhR activation by the intestinal microbiota is involved in the decreased IL-22 production in our model, we determined the concentration of AhR ligands in the feces of our mice. Production of IAA was impaired in feces of $Card9^{-/-} \rightarrow$ GF mice. Using mice hepatoma cell lines containing a stably integrated DRE-driven firefly luciferase reporter plasmid we found that feces from $Card9^{-/-} \rightarrow$ GF mice were defective in their ability to activate AHR, to a similar extent as that for feces from GF mice [17*]. *Lactobacillus reuteri* can catabolize tryptophan to indole derivatives with AhR agonistic activity and there is preliminary evidence that other species can also produce AhR agonists via unknown pathways [2]. In line with these data, culture supernatants of *L. reuteri* and *Allobaculum* sp., two bacteria with decreased abundance in the $Card9^{-/-}$ mice microbiota, strongly activate AhR [17*]. These results suggested that impaired tryptophan metabolism by the microbiota of the $Card9^{-/-}$ mice could be associated with, or even be responsible for, the hypersusceptibility of $Card9^{-/-}$ mice to colitis. In WT mice, bacterial microbiota analysis allowed us to isolate several strains producing high amounts of AhR ligands including three strains of *Lactobacillus* (*L. murinus*, *L. reuteri* and *L. taiwanensis*). In $Card9^{-/-} \rightarrow$ GF mice, susceptibility of colitis, and IL-22 defect were rescued after treatment with AHR agonist (6-formylindolo(3,2-b)carbazole), or inoculation with the three *Lactobacillus* strains with strong AHR agonist activity. These effects were mediated by AHR, as they were abrogated in the presence of an AHR antagonist (CH223191) [17*].

These results showed that impaired tryptophan metabolism in the microbiota of the *Card9*$^{-/-}$ mice lead to defective AhR activation which contributes to the susceptibility of mice to colitis by reduction of IL-22 production.

Impaired AhR activity and tryptophan metabolites in patients with IBD

To determine whether these findings were relevant to human disease, we analyzed fecal samples from individuals with IBD and healthy subjects for their ability to activate AHR. Impaired AhR activity associated with decreased concentrations of IAA and tryptophan were observed in feces of patients with IBD. These patients were also genotyped for an IBD-associated single-nucleotide polymorphism (SNP) within *CARD9* (rs10781499) [22]. We observed that the risk allele was associated with reduced AhR activation by microbiota-derived metabolites extracted from fecal samples. No association was observed among other major IBD-associated SNP, including those in *NOD2* [17*]. These results suggest a connection between IBD, CARD9, and the ability of the microbiota to produce AHR agonists in humans.

Conclusion

These results in human should be confirmed in an independent cohort but indole derivatives, or the microorganisms that produce them, could be used as a supportive therapy in individuals with intestinal dysbiosis to override the defect of tryptophan metabolism by the gut microbiota. In a more general concept, our findings support the notion that abnormal innate immunity, such as defect in CARD9, can shape an altered microbiota, which can then modify the host immune response and amplify the dysbiosis in

a vicious cycle leading to the loss of intestinal homeostasis. Therefore, the respective roles of the host factors and the gut microbiota in IBD pathogenesis cannot be completely distinguished. Thus, dysbiosis should neither be considered a cause nor a consequence of IBD, but both simultaneously.

Key points

- Alteration of intestinal microbiota is observed in IBD.

- CARD9 is one of the identified IBD susceptibility genes.

- CARD9 has a role in shaping the bacterial and fungal gut microbiota and it is required for the production of AhR ligands by the microbiota.

- Impaired ability of the microbiota to catabolize tryptophan into AhR ligands increased sensitivity to colitis by altering the IL-22 signaling pathway.

- Impaired microbial production of AHR ligands is observed in patients with IBD and correlates with an IBD-associated SNP within *CARD9.*

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Conflicts of Interest

None.

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